Cell type-specific endometrial transcriptome changes during initial recognition of pregnancy in the mare

Scaravaggi, Iside ; Borel, Nicole ; Romer, Rebekka ; Imboden, Isabel ; Ulbrich, Susanne E ; Zeng, Shuqin ; Bollwein, Heiner ; Bauersachs, Stefan

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Complete List of Authors:	Scaravaggi, Iside; University of Zurich, Department for Farm Animals, Clinic of Animal Reproduction Medicine Borel, Nicole; University of Zurich, Institute of Veterinary Pathology Romer, Rebekka; University of Zurich, Department for Farm Animals, Clinic of Animal Reproduction Medicine Imboden, Isabel; University of Zurich, Department for Farm Animals, Clinic of Animal Reproduction Medicine Ulbrich, Susanne; ETH Zurich, Institute of Agricultural Sciences, Animal Physiology Zeng, Shuqin; University of Zurich, Department for Farm Animals, Clinic of Animal Reproduction Medicine Bollwein, Heinrich; University of Zurich, Department for Farm Animals, Clinic of Animal Reproduction Medicine Bauersachs, Stefan; University of Zurich, Department for Farm Animals, Clinic of Animal Reproduction Medicine
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Short title: RNA-seq of equine microdissected endometrium samples

Summary sentence: Cell type-specific RNA-sequencing of equine endometrial biopsies from Day 12 of pregnancy and the corresponding day of the estrous cycle revealed distinct gene expression in luminal epithelium, glandular epithelium, and stromal areas, which was compared to RNA-Seq data obtained for whole biopsy samples.

Keywords: Equus caballus, uterus, maternal recognition of pregnancy, laser capture microdissection, RNA-seq

Iside Scaravaggi[1,2], Nicole Borel[3], Rebekka Romer[1], Isabel Imboden[1], Susanne E. Ulbrich[2], Shuqin Zeng[1,2], Heinrich Bollwein[1], and Stefan Bauersachs[1,2]

[1]University of Zurich, Clinic of Reproductive Medicine, Department for Farm Animals, Zurich, Switzerland; [2]ETH Zurich, Animal Physiology, Institute of Agricultural Sciences, Zurich, Switzerland; [3]University of Zurich, Institute of Veterinary Pathology, Zurich, Switzerland

Corresponding author: Stefan Bauersachs

Genetics and Functional Genomics, Clinic of Reproductive Medicine, Department for Farm Animals, University of Zurich, Agrovet-Strickhof, Eschikon 27, EHB E 23.1, CH-8315 Lindau, Switzerland, Phone +41 52 354 91 85, E-mail: stefan.bauersachs@uzh.ch

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Abstract

Previous endometrial gene expression studies during the time of conceptus migration did not provide final conclusions on mechanisms of maternal recognition of pregnancy (MRP) in the mare. This called for a cell type-specific endometrial gene expression analysis in response to embryo signals to improve understanding of gene expression regulation in context of MRP. Laser capture microdissection was used to collect luminal epithelium (LE), glandular epithelium (GE), and stroma from endometrial biopsies from Day 12 of pregnancy and estrous cycle, respectively. RNA-sequencing showed greater expression differences between cell types than between pregnant and cyclic states. Differences between pregnant and cyclic state were mainly found in LE. The comparison with a previous RNA-Seq data set for whole biopsy samples revealed the specific origin of gene expression differences. Furthermore, genes specifically differentially expressed (DE) in one cell type were found, which were not detectable as DE in biopsies. Overall, this study revealed spatial information about endometrial gene expression during the phase of initial MRP. The conceptus induced genes involved in blood vessel development, specific spatial regulation of the immune system and growth factors, and regulation of prostaglandin synthesis, transport, and prostaglandin receptors, specifically *PTGFR* in context of prevention of luteolysis.

Introduction

In many mammals, particularly those with late implantation, maternal recognition of pregnancy (MRP) is a fundamental step for successful gestation. The conceptus must signal its presence to the maternal organism to prevent luteolysis which would lead to embryo loss (Allen 2001b). This mechanism has been unraveled to a large extent in most of the large domestic animal species but not in equids (Allen 2001a; Allen and Wilsher 2009). The pig embryo, like the equine conceptus, secretes considerable amounts of estrogen which has been proven as responsible for MRP in the sow but not in the mare (Wilsher and Allen 2011; Bazer 2013).

The major steroid produced during early pregnancy by the equine conceptus is 17 alpha-hydroxyprogesterone, which also could be involved in prevention of luteolysis and conceptus development (Betteridge 2000). Increased expression of potential receptors for this specific progesterone metabolite has been shown in the endometrium on day 12 of pregnancy (Merkl *et al.* 2010). The same study investigating the effects of estrogen found out that intrauterine administration of plant oils at day 10 after ovulation inhibits luteolysis in mares (Wilsher and Allen 2011). Although the exact mechanism is unclear, it is likely that the lipids contained in the studied plant oils modulate the synthesis or release of prostaglandins from the endometrium (Wilsher and Allen 2011).

The ruminant embryo produces a specific type I interferon, interferon tau (IFNT), which prevents the release of luteolytic prostaglandin $F_{2\alpha}$ (PGF2a) (Roberts 1996). Trophoblast IFNT prevents estrogen-induced increases in oxytocin receptor (*OXTR*) gene expression in the endometrium by repression of estrogen receptor alpha (*ESR1*) expression in the endometrial luminal epithelium (Spencer and Bazer 1996). The equine conceptus does not produce alpha or omega interferon molecules, but delta interferons (Baker *et al.* 1991; Cochet *et al.* 2009). They have been suggested to play a role in embryo-maternal interaction but not in MRP in the mare (Cochet *et al.* 2009). Similar to ruminants, endometrial concentrations of OXTR determine uterine PGF2a secretion in cyclic mares and endometrial OXTR concentrations as well as receptor affinity were reduced in early pregnancy by the presence of the conceptus (Sharp *et al.* 1997; Starbuck *et al.* 1998). However, the pulsatile endometrial release of PGF2a into the peripheral circulation, which is the initial signal for luteolysis (Aurich 2008), is someway suppressed by the presence of the equine conceptus and/or its secretions (Goff *et al.* 1987; Wilsher and Allen 2011).

A particularity that only the equine embryo is showing, is the self-induced mobility throughout the entire uterus between days 6 and 17 after ovulation by peristaltic contractions of the uterus in response to prostaglandins derived from the embryo (McDowell *et al.* 1988; Stout and Allen 2001; Allen and Wilsher 2009). One of the conceptus prostaglandins is PGF2a having a local function in stimulating the myometrial contractility but also the rapid expansion of the early

equine blastocysts (Stout and Allen 2001; Stout and Allen 2002). Studies in sheep have shown an important role of prostaglandins in conceptus development and regulation of endometrial function (Dorniak *et al.* 2011; Dorniak *et al.* 2012). After the systemic administration of the synthetic prostaglandin inhibitor, flunixine meglumine, conceptus movement is abolished resulting in luteolysis (Stout *et al.* 2000).

In context of the migration, the equine conceptus has another unique property that is its typical spherical shape given by the glycoprotein capsule (Rivera Del Alamo *et al.* 2008; Allen and Wilsher 2009) present between days 7-21 of gestation (Betteridge 2000). This capsule makes the conceptus to resist the myometrial forces that move it through the uterus (Stout *et al.* 2005). McDowell et al. first demonstrated the importance of embryo mobility in conjunction with MRP by ligation of one uterine horn or one uterine horn and the uterine body which led to return to estrus (McDowell *et al.* 1988). Later on, Rivera Del Alamo et al. demonstrated that the introduction of an intrauterine device (IUD, water-filled plastic ball, diameter 20 mm) induced a prolonged luteal phase in 75% of the mares. The mechanism of action is still unclear but it was suggested that the IUD mimics an embryo preventing endometrial cells from releasing PGF2a due to the close contact of the IUD with the endometrium (Rivera Del Alamo *et al.* 2008). In general, mechanotransduction or mechanosensation has been shown to be involved in molecular mechanisms governing epithelial and endothelial cell development and physiology and is mediated via interactions between extracellular matrix, cellular junctions, and the cytoskeleton (Ohashi *et al.* 2017; Sluysmans *et al.* 2017). Moreover, in chondrocytes a mechanism for mechanotransduction has been shown to involve integrin signaling resulting in a reduction of interleukin 1beta (IL1B)-induced nitric oxide and prostaglandin E2 release by decreasing the expression levels of nitric oxide synthase 2 (*NOS2*, iNOS) and prostaglandin-endoperoxide synthase 2 (*PTGS2*, COX-2).

To decipher transcriptomic and proteomic changes in the endometrium, uterine fluid, and conceptus during the time of MRP in the mare a number of “omics” studies have been performed (Hayes *et al.* 2008; Klein *et al.* 2010; Merkl *et al.* 2010; Hayes *et al.* 2012; Klein 2015; Klohonatz *et al.* 2015). Endometrial gene expression changes in response to the presence of a conceptus have been studied between Days 8 and 18 by the use of DNA microarrays and RNA sequencing (RNA-Seq). Changes in the endometrial transcriptome have been detected as early as Day 12 of pregnancy (Merkl *et al.* 2010). Overall, a very complex endometrial response to the presence of the conceptus was observed. The identified gene sets had very heterogeneous biological functions that could reflect i) a response to different embryonic signals and/or ii) differential gene expression in different compartments of the endometrium, such as luminal epithelium (LE), glandular epithelium (GE), and stroma (Klein *et al.* 2010; Merkl *et al.* 2010). For example, many

genes differentially expressed between pregnant and cyclic endometrium are known to be regulated by estrogens. Although these endometrial transcriptome studies revealed many new insights into gene expression changes in response to the presence of the equine conceptus, direct conclusions on the equine embryonic pregnancy recognition signal and the mechanisms of MRP in the horse could not be drawn from the obtained results. Thus, these findings call for a deeper analysis of cell-type specific gene expression of the endometrium in response to the equine embryo, which could be the key to understand the mechanism of MRP in the horse.

The aim of the present study was to analyze the cell type-specific endometrial response to the presence of a conceptus at the level of gene expression on Day 12 of pregnancy based on the isolation of distinct endometrial compartments by the use of Laser Capture Microdissection (LCM). Day 12 of pregnancy was chosen based on our results from a previous study (Merkl *et al.* 2010) and in order to compare the obtained cell type-specific results to those obtained from the analysis of entire biopsy samples.

Material and Methods

Sample collection and Experimental Design

The sample collection for RNA-Seq of whole biopsy samples has been described previously (Merkl *et al.* 2010). Endometrium biopsies for the isolation of specific endometrial cell types were collected from 6 healthy cycling warmblood mares belonging to the University of Zurich, Clinic of Reproductive Medicine, Switzerland. The mares were monitored for signs of estrus using a LOGIQ e ultrasound equipment (GE Healthcare, Glattbrugg, Switzerland). When a follicle of at least 35 mm diameter was detected in conjunction with an uterine edema, a single dose of 1500 IU human choriogonadotropin (hCG) was applied i.v. to induce ovulation (day -2). The mares were randomly assigned to one of the experimental cycles: control cycle or pregnancy. If assigned to the pregnancy cycle the mare was inseminated with fresh cooled semen one day before ovulation (day -1). For all samples used for the pregnant group, pregnancy was confirmed by ultrasound examination on day 12 after insemination before sample collection when in case of pregnancy the presence of a conceptus can be reliably detected. To induce luteolysis at the end of the experiment, PGF2a was applied on day 12. During the experimental days (-2 to 12) ultrasound images of both uterus and ovaries were recorded and blood samples for measurement of progesterone were collected. Blood samples were centrifuged, blood plasma collected and stored at -20°C until assay. On day 12, endometrial samples were obtained by performing a uterine biopsy. After a longitudinal cut of the biopsy, one half was fixated in formalin for histological examination and the other half was snap-frozen in a cryo-embedding matrix in liquid nitrogen and stored at -80°C.

All the experiments with animals were conducted with the permission of the veterinary inspection office of the Kanton Zurich (permission no. 24/2014) and the degree of severity corresponded to grade 1.

Histological analysis of endometrial biopsies

The endometrial biopsy samples where first evaluated macroscopically and then fixed in a 4% formalin solution for 24 hours. Subsequently, the endometrial samples were embedded in paraffin blocks (Sakura Tissue® Tek® VIP, GMI, Ramsey, MN, USA). The blocks were rinsed with water for 10 minutes, dehydrated in a graded series of alcohols for 5:15 hours (70%, 80%, 96%, 100%), cleared with xylene for 3:30 hours and embedded in paraffin for 3 hours. Then, 2 µm sections were prepared and stained with haematoxylin and eosin according to standard procedure. The histological sections where evaluated and categorized according to Kenney and Doig (Kenney and Doig 1986).

Laser capture microdissection (LCM)

Stained frozen sections (10 µm) were prepared using a microtome cryostat CryoStar NX50 (Histocom AG, Zug, Switzerland) on PEN-Membrane Glass Slides®, and Arcturus® HistoGene® Frozen Section Staining Kit (Applied Biosystems). The collection of luminal epithelium (LE), glandular epithelium (GE) and stromal areas (S) was performed using an ArcturusXT® Laser Capture Microdissection instrument.

RNA isolation, quantification and quality control

RNA isolation was performed with the Arcturus PicoPure™ RNA Isolation Kit (Thermo Fisher Scientific, Darmstadt, Germany). Concentration of the RNA was measured with a *Quantus™* Fluorometer (*Promega*; sensitivity 390 pg RNA/ml using QuantiFluor® RNA Dye). Quality was analyzed by the use of the *Agilent 2100* Bioanalyzer (Eukaryote Total RNA Pico Assay), which estimates the concentration of the RNA sample and provides a quality score (RNA integrity number, RIN).

Illumina RNA-Sequencing and data analysis

For RNA derived from whole biopsy samples, the mRNA-Seq sample preparation kit (Illumina, San Diego, USA) was used for preparation of RNA-Seq libraries as described recently (Samborski *et al.* 2013). For each group (Day 12 pregnant and non-pregnant cyclic mares), 4 RNA-Seq libraries were prepared corresponding to samples from 4 mares, respectively. Sequencing of the libraries was conducted on an Illumina Genome Analyzer IIx system. Single-end reads (76 bp) were generated using Cluster generation single read, cBot kit and Cycle sequencing kit v4 (Illumina). Each RNA-Seq library was analyzed on one lane of a single-read flow cell (in total 8 lanes).

For the generation of RNA-Seq libraries from LCM samples, the Ovation® Single Cell RNA-Seq System (NuGEN Technologies, Inc., European office, Leek, The Netherlands) was used starting from 1 ng total RNA. Since for a number of libraries the concentration of the obtained cDNA fragments was too low (Agilent Bioanalyzer DNA High Sensitivity assay), an additional amplification was performed with the KAPA HiFi Library Amplification Kit (Axon Lab AG, Baden, Switzerland). Single-end reads (125 bp) were generated on an Illumina HiSeq 2500 instrument running multiplexed libraries on two lanes of one flow cell.

The obtained sequence reads (Fastq files) of both studies were analyzed with the same data analysis pipeline on a locally installed version of Galaxy (Giardine *et al.* 2005) essentially as described recently (Kradolfer *et al.* 2016). Potential PCR duplicates were removed using the tool FastUniq (Galaxy Version 1.1). The most current GFF3 annotation file from NCBI was used for

mapping and assignment of mapped reads to genes. Library sizes (reads mapped to annotated genes) were on average 598,000 (ranging from 138,000 to 1,467,000). All analysis steps after generation of the read count table were performed using the BioConductor package EdgeR (Robinson *et al.* 2010) according to the EdgeR users guide including the identification of differentially expressed genes (DEGs). Due to the low number of biological replicates in some LCM sample groups and the suboptimal RNA quality, a rather explorative approach was used to identify DEGs and to reduce the number of false positives. This consisted of two approaches; i) filtering of DEGs based on read counts/counts per million (CPM) of individual samples, and ii) checking expression in LCM samples of genes differentially expressed (DE) in the complete biopsy samples to assign spatial gene expression information. The filtering was performed by checking the CPM of each DEG revealed by the EdgeR analysis if similar expression was detected in each sample of the group with higher expression and expression was lower in all samples of the group with on average lower expression. For example, if read counts for C_S_B, C_S_M, P_S_M, P_S_U (C, P: control, pregnant; S: stroma; last letter: code for the mare) were 1, 0, 534, 3, this gene was not considered as DEG. Also, if read counts were 128, 2, 156, 98, such genes were removed from the DEG list. Also, for LCM sample groups with more replicates, a similar filtering was used. In the second approach, the probable location of gene expression of DEGs found in the whole biopsy was indicated by the expression data from the LCM samples, i.e., if CPM were very low or 0 in two cell types and higher in the third cell type. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession numbers GSE112236 (LCM samples) and GSE112237 (biopsy samples).

Venn diagrams were generated with the web tool jvenn (Bardou *et al.* 2014). Integrated analysis of different functional databases was done using the “Functional annotation clustering” and “Functional Annotation Charts” tool of the Database for Annotation, Visualization, and Integrated Discovery (DAVID 6.8) (Dennis *et al.* 2003). Graphical illustration of overrepresented gene ontologies and other functional categories was produced with the Cytoscape v3.0.0 application ClueGO v2.0.3 (Bindea *et al.* 2009). Hierarchical clustering of DEGs was performed with the HCL tool of Multi Experiment Viewer (MeV) v4.8.1 (Saeed *et al.* 2003) based on mean-centered normalized log2 transformed read counts (normalized value of a sample minus average of all normalized values).

Results

Histological analysis of endometrial biopsies and serum progesterone concentrations

Signs of inflammation and grade of endometrial fibrosis are the markers used to estimate the mare's ability to conceive and maintain a pregnancy. The endometrium biopsies collected for LCM were graded between I (normal endometrium) and II A (moderate inflammation and multifocal-diffuse fibrosis).

During estrus, serum progesterone (P4) concentrations in serum were always below 1 ng/ml (Supplemental Table 1). Except for two values, the P4 concentration on the day before ovulation were still below 1 ng/ml. During diestrus, concentrations were between 5 and 20 ng/ml with high individual differences.

RNA isolation from cells isolated by Laser Capture Microdissection and RNA sequencing

Cells from luminal epithelium (LE), glandular epithelium (GE) and stromal (S) areas were collected by Laser Capture Microdissection (LCM) from stained frozen sections prepared from endometrial biopsies collected on Day 12 of pregnancy and Day 12 of the estrous cycle, respectively. The RNA yield from the cells isolated by LCM was between 0.5 and 18 ng. The samples used for RNA-Seq showed partial degradation but were at least in a similar quality range (RIN 5-7). RNA-Seq libraries were produced using samples from 5 mares, i.e., a non-pregnant control cycle and a pregnancy cycle of each mare corresponding to 30 RNA samples. For most of the 30 samples cDNA fragments could be amplified in sufficient amounts for sequencing. Twenty-one out of 30 libraries were selected for sequencing based on the results of the library product analysis. Altogether, data derived from 19 samples was found to be useful for statistical analysis of gene expression (LE pregnant: n=3, LE cyclic: n=5, GE pregnant: n=4, GE cyclic: n=3, stroma pregnant: n=2, stroma cyclic: n=2). A pairwise distance heatmap illustrating the correlation of the expression profiles of the samples (Figure 1A) and a principal component analysis (Figure 1B) indicated that gene expression differences between LE, GE and stroma were greater than differences between pregnant and cyclic samples of the respective cell types. Since the distance of LE samples to the stroma and GE samples were greatest for principal component 1 the gene expression in LE was found as most distinct compared to the other cell types. The most significant differences between pregnant and cyclic mares were observed for LE samples.

Comparison of RNA-Seq of whole biopsy samples and cell type-specific samples collected by LCM

The number of detectable genes (reaching a threshold of read counts) was compared between the data sets for whole biopsies and LCM samples (Figure 2). In the RNA-Seq analysis of the biopsies, 3806 genes were detected which were not detectable in the LCM samples. Altogether, 175 genes were detectable in LE, GE, and/or stroma but not in the analysis of the biopsy samples (Figure 2). Interestingly, the highest number of specifically expressed genes in the LCM data sets was obtained for LE. The lowest number of detectable genes (6880) was observed for stroma. A relatively high number of genes was detected in both epithelia but not in the stromal area (2725 genes).

Analysis of differential gene expression

Because of the effects of the low RNA concentration and the partial degradation of the RNA samples on the quality of the RNA-Seq data (increased variation between individual samples), a rather explorative strategy for identification of DEGs had to be applied. The first approach was based on a statistical analysis of the RNA-Seq data for the LCM samples and a rigorous filtering of the obtained DEGs to minimize false positive results. Furthermore, a comparison between the cell type-specific data and the available RNA-Seq data set for endometrium biopsy samples from day 12 of pregnancy and cycle was performed (Bauersachs and Wolf 2012; Bauersachs and Wolf 2015). Here, the results from the cell type-specific data were used to identify DEGs obtained from the analysis of complete endometrium biopsies of cyclic and pregnant mares which are specifically expressed in the endometrial compartments. The numbers of identified DEGs for endometrial biopsy analysis and for the analysis of specific endometrial cells are shown in Table 1. The Venn diagram in Figure 3 shows the overlap of DEGs between whole biopsy, LE, GE and stroma. For 135 out of the 502 DEGs obtained from the analysis of biopsy samples, the localization of differential gene expression could be determined. Furthermore, 185 DEGs were only found in the analysis of samples isolated by LCM, most of them showing specific expression in LE or stroma. The number of obtained DEGs in GE was low (42 genes), whereas the analysis of LE (165 genes) und stroma (154 genes) revealed a higher number of DEGs. A hierarchical cluster analysis of the relative gene expression (mean-centered) across all LCM samples for the DEGs identified by the first approach (statistical analysis of LCM results and DEGs filtering) showed that many of the DEGs are specifically expressed or downregulated in the respective cell type (Supplemental Figures 1-3). A more detailed view of the DEGs for the LCM samples is shown in the Venn diagram in Figure 4. Whereas the number of genes with higher (upregulated) or lower expression (downregulated) in samples collected from pregnant

mares was similar for LE and GE, the majority of DEGs in stroma showed higher expression for pregnant mares (upregulated). Also for the analysis of biopsy samples more upregulated than downregulated genes were found. In addition, the comparison of LE and stroma revealed some genes with opposite regulation, i.e. higher expression in stroma of pregnant mares but lower expression in LE compared to non-pregnant cyclic controls.

Overrepresentation analysis of differentially expressed genes

A functional classification of the obtained DEGs for the single endometrium compartments and for the complete biopsy data set was performed to identify overrepresented functional categories. DAVID Functional Annotation Clustering (Dennis *et al.* 2003) was performed for genes up-regulated or down-regulated in endometria from day 12 pregnant mares compared to day 12 cyclic controls (Supplemental Table 3). For all cell types and complete biopsy samples, less significant annotation clusters were obtained for down-regulated genes. Genes up-regulated in LE were enriched for processes/functions associated with secretion, development, signaling, immune response, and proteolysis and cellular components related to vesicles and membrane. Overrepresentation of down-regulated genes in LE was only found for a few groups of functional categories with relatively low numbers of assigned genes. These categories were related to retinoid metabolism, cell motility/migration, and signaling. Due to the low number of DEGs for GE, no significant annotation clusters were obtained for GE and Functional Annotation Chart analysis was done instead. Functional terms enriched for genes up-regulated in GE represented secretory processes, reproductive processes, and signaling. Some categories involved in secretory processes were also enriched for genes down-regulated in GE. The majority of the other enriched functional categories was related to various metabolic and catabolic processes. For stroma, genes identified as up-regulated in samples from pregnant mares were overrepresented for a variety of processes/functions, such as wound healing, secretion, cell migration, development, signaling and cell communication, metabolism and biosynthesis, angiogenesis, cell adhesion, and gene expression. Enriched cellular components were related to vesicles and membrane, focal adhesion and cell junction, and contractile fiber. Genes down-regulated in stromal areas of endometria from pregnant mares were enriched for processes involved in lipid and DNA metabolism, cytoskeleton, and reproduction. In the complete biopsy, most of the overrepresented functional categories were also obtained for the LCM samples. Some processes/functions were not found as overrepresented for the LCM samples, e.g., a number of immune function processes, unsaturated fatty acid and icosanoid metabolic process, ion channel, and terms related to blood vessel development. Genes down-regulated in complete biopsies were mainly enriched for metabolic processes and extracellular matrix.

To perform a comparison of the functional annotation results, all functional terms to which genes up-regulated in pregnant mares could be assigned were analyzed between the different cell type data sets and the biopsy data set (Supplemental Table 4). Most of the functional terms related to blood vessel development and angiogenesis were significantly enriched only for the biopsy sample. Many terms containing genes involved in immune response and response to various factors were enriched for the complete endometrial biopsy. A number of those terms was also enriched in LE and/or stroma. The same was found for terms related to proliferation, cell death, and metabolic processes. Of those, some specific terms were only enriched for LE, such as terms related to protein glycosylation. Cell adhesion and junction terms were mainly found as overrepresented for the complete biopsy. Terms for extracellular matrix and connective tissue development were enriched for biopsy and LE. Secretion and extracellular vesicle were found as enriched for biopsy and LCM samples. Overall, the comparison revealed the localization of differential gene expression for overrepresented functional terms. Furthermore, some overrepresented functional terms were found which were not significant for the complete biopsy sample but for a specific cell type.

Finally, DEGs were compared to a number of gene lists assigned to pathways involved in prostaglandin synthesis and regulation, steroid hormone biosynthesis, and estrogen, oxytocin, prolactin, interferon and IL-1 signaling (Supplemental Table 5). Most of the assigned genes were found as upregulated, but mainly only in the analysis of complete biopsy samples. For the genes found as differentially expressed in the LE 7 were downregulated and 5 upregulated, genes in GE and stroma were mostly upregulated. The genes assigned to PG metabolism and signaling were involved in the release of PG precursor molecules (*PLA2G2A* down in LE, *PLA2G4F* up in biopsy) from the plasma membrane, PG transport (*ABCC1* up in LE and GE, *SLCO2A1* up in biopsy), or function as PG receptors (*PTGER4* up in biopsy, *PTGFR* down in biopsy). In addition, two annexin genes were found, *ANXA1* downregulated in stroma and *ANXA2* upregulated in LE. In agreement with our previous findings (Merkl *et al.* 2010), Oxytocin receptor (*OXTR)* mRNA was upregulated (stroma) and estrogen receptor alpha (*ESR1*) mRNA downregulated in the complete biopsy, but upregulated in stroma.

Discussion

The motivation to study gene expression in individual endometrial cell types was based on previous findings in the mare and other species indicating distinct differences in gene expression between endometrial compartments in response to embryonic signals and during the estrous cycle (Niklaus and Pollard 2006; Merkl *et al.* 2010; Bauersachs and Wolf 2012; Filant and Spencer 2013; Field *et al.* 2015; Brooks *et al.* 2016; Rosario *et al.* 2016). Furthermore, the endometrial epithelia, particularly the LE, are thought to play important biological roles in conceptus survival, growth, and implantation in mammals showing non-invasive placentation (Spencer *et al.* 2007; Brooks *et al.* 2016; Spencer *et al.* 2016). Since the percentage of LE in an endometrial biopsy sample is relatively low, many specific changes in gene expression probably remain undetectable when analyzing whole endometria due to dilution and averaging effects. Furthermore, with respect to data analysis and interpretation, it is difficult to draw conclusions on involved pathways if the localization of gene expression regulation is unknown.

The present study revealed that LE, GE and stroma of the equine endometrium have specific transcriptome profiles and respond specifically to conceptus signals which is in agreement with findings in other species (Niklaus and Pollard 2006; Field *et al.* 2015; Brooks *et al.* 2016). Interestingly, with respect to differential gene expression the difference between LE and GE seems to be greater than between GE and stroma. The main response to the presence of a conceptus, i.e. highest number of DEGs was found in the LE. In contrast, more DEGs were found in GE than in LE in the course of the preimplantation phase in a recent study in sheep (Brooks *et al.* 2016). Although in a number of studies, transcriptome analysis of endometrial LCM samples has been performed, so far, no study compared the three main compartments and complete endometrial samples.

As expected, a higher number of detectable genes was present in the data set for complete endometrial biopsies. Since the preparation of RNA-seq libraries started from only 500 pg of total RNA (corresponding to 50 cells or less) and the quality of the RNA from LCM samples was lower compared to the biopsy samples, some transcripts present in very low copy numbers probably got lost during library preparation. Nevertheless, expression of some genes was only detectable in the LCM samples, which could be due to specific expression in certain cell types, making detection in a whole endometrium biopsy very difficult because of the dilution effect. In addition, the LCM samples for stroma were cut from regions without visible blood vessels, why the biopsy samples may contain transcripts specific for endothelial cells and blood vessel associated immune cells which are not present in the LCM samples.

Despite the limitation due to the suboptimal quality of the RNA isolated from the LCM samples and the low number of replicates, the obtained data could be used to identify cell type-specific differential gene expression by applying stringent filtering criteria. This revealed localization of differential gene expression in the endometrium and identification of DEGs not detectable in complete biopsy samples because of averaging and dilution effects. Even opposite regulation of gene expression was found for a number of genes in LE and stroma. So far, this has not been reported. In a few studies, cell type-specific endometrial expression of transcripts or proteins has been studied during the preimplantation phase for selected targets (Spencer *et al.* 2008; Ka *et al.* 2009; Silva *et al.* 2011; Seo *et al.* 2014a).

The data analysis of the current study revealed enrichment of specific functional categories and biological processes for the investigated cell types, thereby localizing regulatory processes in response to the conceptus to functional compartments of the endometrium. In the following, the results obtained for functional groups of genes potentially important for conceptus-endometrial interactions are discussed. However, although this study contains a lot of interesting new information, due to limitations in the number of biological replicates and the quality of RNA isolated from LCM samples, it is rather to see as proof-of-concept study with an explorative character. The reader should be aware that before starting more in-depth functional studies on interesting genes identified in this study, these findings should be first validated.

Genes related to angiogenesis and blood vessel development. In our previous study, one predominant complex of overrepresented categories was related to angiogenesis and blood vessel development (Merkl *et al.* 2010). The analysis of the DEGs for the complete biopsy sample confirmed this finding. The DEGs for the individual cell types did not show highly significant enrichment for these terms, except for LE where 'vasculature development' was significantly enriched with 11 genes. In contrast to the DEGs assigned to 'vasculature development' identified in the complete biopsy samples, the DEGs in LE were not classical angiogenesis or vessel development genes. Based on their known functions, most of those genes have a more indirect role in stimulation of vascular development and differentiation suggesting a paracrine effect on blood vessels. For example, angiopoietin like 4 (ANGPTL4), fibroblast growth factor 9 (FGF9), C-X-C motif chemokine ligand 17 (CXCL17), nephroblastoma overexpressed (NOV), and Wnt family member 7B (WNT7B) could be factors expressed in LE and having effects on vasculature development. In the mouse, expression of Angptl4 mRNA has been shown to increase in implantation segments during decidualization and in endometrial stromal cells undergoing decidualization in vitro (Scott *et al.* 2012). ANGPTL4 has been shown to function in modulation of vascular permeability, angiogenesis, and inflammatory signaling

(Guo *et al.* 2014) but also in regulation of triglyceride metabolism (Mattijssen and Kersten 2012). FGF9 is affecting blood vessel development and functions indirectly via induction of smooth muscle cell proliferation and wrapping of microvessels (Agrotis *et al.* 2004; Frontini *et al.* 2011). CXCL17 has been shown to induce the production of proangiogenic factors such as vascular endothelial growth factor A (VEGFA) from treated monocytes (Lee *et al.* 2013). Increased expression of galectin 3 binding protein (*LGALS3BP*) mRNA was detected in LE similar to findings in cattle (Bauersachs *et al.* 2006). Expression of this gene has been described in association with immunomodulatory effects (Laubli *et al.* 2014). Furthermore, *LGALS3BP* expression in tumor cells has been shown to induce *VEGF* expression and to stimulate blood vessel formation (Piccolo *et al.* 2013). NOV has been shown to directly stimulate endothelial cells and to induce angiogenesis in vivo (Lin *et al.* 2003). WNT7B could also have an effect on blood vessel formation and remodeling in the endometrium based on findings in other tissues, e.g., in renal medullary capillary development (Roker *et al.* 2017), and furthermore, the proposed role of the WNT signaling pathway in placenta formation (Cross *et al.* 2003). The DEGs identified in the complete biopsy samples and assigned to 'vasculature development' contained a number of typical genes involved in angiogenesis and blood vessel remodeling and formation, such as members of the vascular endothelial growth factor pathway, angiopoietin system, structural molecules of endothelial cells, and angiogenesis-related transcription factors. Since the samples isolated by LCM did not contain blood vessels (stromal areas containing visible vessels were not selected for cutting), the differential expression of these genes is most likely located in endothelial cells. Furthermore, the increase of expression of endothelial markers suggests an increase of vascularization in response to the presence of the conceptus. In agreement with this idea are previous findings that revealed a role of the conceptus in directing vasculogenesis and endometrial vascular perfusion during early pregnancy (Silva *et al.* 2005; Silva *et al.* 2011).

Genes involved in immune response. Another group of highly enriched functional categories for genes upregulated in complete endometria samples was related to immune response. "Inflammatory response" was significantly enriched for complete biopsy but also found as overrepresented for LE. The corresponding genes upregulated in LE were mainly involved in negative regulation of inflammatory response. The products of *C5orf30* (Muthana *et al.* 2015), *CXCL17* (Oka *et al.* 2017), *NOV* (Liu *et al.* 2014), *TNFRSF21* (Liu *et al.* 2001), and the complement inhibitors CD55 (Palomino *et al.* 2013), SERPING1 (Beinrohr *et al.* 2011) have been shown to modulate and suppress immune response. In the context of induction of immune tolerance, *CD200* (Clark *et al.* 2015) has been described as tolerance-signaling molecule in humans and was found as expressed in LE and stroma and upregulated in the complete biopsy

in the present study. In contrast, the genes upregulated in the complete biopsy were predominantly known as associated with activation of immune response such as complement components, activating chemokines, and genes expressed in activated cells of the specific immune system. Interestingly, upregulation of the mRNA for TNF receptor superfamily member 18 (*TNFRSF18*) was found in complete biopsy samples with very low but increased expression in samples from pregnant mares. The gene *TNFRSF18* is known to be expressed at high levels in activated T cells and regulatory T cells (Ronchetti *et al.* 2015). Overall, based on the genes found as upregulated in response to the presence of an embryo pregnancy, the immune system is modulated in a way to inhibit inflammatory response and induce tolerance.

Genes with probable functions in interaction with the conceptus. Genes involved in positive regulation of cell proliferation and negative regulation of apoptosis were overrepresented for LE (upregulated genes). Many of the genes involved in cell proliferation are known to function as autocrine or paracrine growth factors or growth factor receptors. The genes *FGF9, WNT7B*, and *NOV* were already discussed above in context of their potential role on blood vessel development. In addition to its effects on angiogenesis, FGF9 has been identified as an autocrine E2-stimulated endometrial stromal growth factor (Tsai *et al.* 2002). In the pig, FGF9 protein was localized in the glandular epithelium on Day 14 of pregnancy and suggested to function as an embryonic growth factor (Østrup *et al.* 2010). In equine endometrium, expression of *FGF9* was mainly found in LE derived from Day 12 pregnant mares. The gene *FGFR2* encoding a high-affinity FGF9 receptor, was expressed in LE and GE. It is very likely that *FGF9* expression in the mare is also induced by E2 from the conceptus like in the pig and by ovarian E2 in humans, but has a distinct localization and has autocrine effects on the LE and/or paracrine effects on the GE as well as on the conceptus that has been shown to express *FGFR1-4* mRNAs (de Ruijter-Villani *et al.* 2013). Insulin like growth factor binding protein 3 (*IGFBP3*) mRNA expression has been shown to be upregulated by P4 in LE of ovine endometrium (Satterfield *et al.* 2008). Secretion of IGFBP3 by human endometrial stromal cells was found to be stimulated in co-culture with mouse embryos suggesting a regulation by embryonic signals (Liu *et al.* 1995). In our study, *IGBFP3* was mainly expressed in LE and a significant upregulation was found for the whole biopsy samples. Indian hedgehog (*IHH*) expression is important for uterine P4-mediated paracrine signaling in context of stromal decidualization and embryo implantation (Wetendorf and DeMayo 2012). Interestingly, in equine endometrium, *IHH* expression was found in all three cell types, but in LE only in samples derived from pregnant endometrium. Expression of stanniocalcin (*STC1*) mRNA, encoding a protein involved in regulation of intracellular calcium and phosphate (Song *et al.* 2006), was found in all three cell types but was strongest in LE. In the pig, *STC1* has been described as a marker for

implantation exclusively expressed in the luminal epithelium (Song *et al.* 2009) whereas in sheep expression was predominantly detected in the endometrial glands after Day 16 of pregnancy (Song *et al.* 2006). In the mouse uterus, *STC1* gene expression is confined to the LE in the non-pregnant state and shifts to mesometrial stromal cells bordering the uterine lumen after implantation suggesting a role in the process of decidualization (Stasko *et al.* 2001). Based on its specific localization of expression at the embryo-maternal interface, the secreted STC1 protein could have effects on the conceptus as well as on other endometrial cells. The distinct differences in *STC1* gene expression compared to other species could indicate a specific function in regulation of conceptus attachment and implantation, which occurs much later in the mare.

With respect to the very long preimplantation phase in the horse, cell adhesion-related gene expression in the LE is particularly interesting. In agreement with a recent study on mucin 1 (MUC1) expression (Wilsher *et al.* 2013), *MUC1* and *MUC3* mRNA was mainly detected in LE and increased in samples from Day 12 pregnant mares.

Genes related to prostaglandin metabolism and signalling. Annexin proteins are involved in the regulation of prostaglandin production. In our study annexin A1 (*ANXA1*) was downregulated in stroma and *ANXA2* upregulated in LE. In a recent study, annexin proteins (*ANXA1*, *ANXA2*, *ANXA5*) were found in uterine flushing on day 14-16 of pregnancy in sheep (Romero *et al.* 2017). Annexin A2 protein has been found as increased in porcine endometrium on Day 12 of gestation compared to Day 12 of the estrous cycle (Moza Jalali *et al.* 2016). Furthermore, ANXA2 has been shown to be involved in embryo attachment to the endometrium in the mouse and in humans (Garrido-Gomez *et al.* 2012; Wang *et al.* 2015). Expression of *ANXA2* mRNA was detected in all three cell types in equine endometrium but specifically increased in LE. Since ANXA2 is a multifunctional protein, its role during early pregnancy in the mare is unclear. It can be speculated that it is involved in the regulation of PG synthesis. The finding in human endometrium that ANXA2 and RhoA are co-localized with the F-actin network in epithelial cells (Garrido-Gomez *et al.* 2012) and the role of Rho kinases and actin cytoskeleton in mechanosensation, could be a link of sensing mechanical stimuli from the migrating conceptus to the regulation of PG synthesis.

In a recent study on endometrial phospholipase A2 isoform expression in equine endometrium, *PLA2G4A* and *PLA2G2A* expression was negatively correlated with progesterone (P4) concentrations (Ababneh and Troedsson 2013). Endometrial PLA2 gene expression is probably involved in controlling PGF2a production by releasing PG precursor molecules from the plasma membrane. In our study we found *PLA2G2A* downregulated in LE and *PLA2G4F* upregulated in

the complete biopsy, suggesting a distinct local regulation of PLA2 gene expression. In addition to the release of PG precursors from the membrane, PG transport plays an important role in PG regulation. The mRNAs for the prostaglandin transporter genes *ABCC1* and *SLCO2A1* have been shown to be expressed in porcine endometrium during the estrous cycle and early pregnancy (Seo *et al.* 2014b; Jang *et al.* 2017). The expression of *ABCC1* in porcine endometrium was mainly localized in LE and GE (Jang *et al.* 2017), whereas *SLCO2A1* expression was found in LE and blood vessels (Seo *et al.* 2014b). In equine endometrium, upregulation of *SLCO2A1* expression was so far only found on day 22 of pregnancy (Atli *et al.* 2010). In our study, *ABCC1* expression was significantly increased in the whole biopsy samples. The data from LCM samples revealed expression in LE and GE with higher values in samples from day 12 of pregnancy similar to the expression pattern in the pig. The transcript for *SLCO2A1* was also significantly increased in the whole biopsy samples, but not detectable in the LCM samples supporting that endometrial *SLCO2A1* expression is mainly located in blood vessels.

Regulation of expression of classical genes involved in PGE2 and PGF2a synthesis, such as *PTGS2* (also known as *COX2*) and PG synthases was not found on Day 12 of pregnancy. Only *PTGIS* was upregulated in the whole biopsy samples. In equine endometrium, PTGIS protein expression was detected in LE and GE during mid-luteal phase (Rebordão *et al.* 2017). In the LCM samples, *PTGIS* mRNA expression was also found in LE and GE with higher values in samples collected from Day 12 pregnant mares (not significant). However, the specific functional of *PTGIS* in the endometrium is not known.

The mRNA for the hyaluronic acid receptor CD44 was downregulated in LE LCM samples and upregulated in stroma and complete biopsy of Day 12 pregnant mares. CD44 is involved in a variety of processes. With respect to PG metabolism, CD44 signalling has been found to activate PTGS2 and generate VEGF resulting in increased proliferation of endothelial cells (Murphy *et al.* 2005). The results found in this previous study are in agreement with the localization of *CD44* expression observed in our study. Since regulation of *PTGS2* at the level of transcription was not observed, regulation at the protein level could be possible. A closer look at the RNA-seq data revealed that *PTGS2* expression is low in whole biopsy samples and in LCM samples only detectable in LE. The expression of other PG synthases such as *PGFS* and *PGES3* was also detected, but in all endometrial compartments.

Similar to findings for Days 14 and 21 (de Ruijter-Villani *et al.* 2015), PGF2a receptor mRNA (*PTGFR*) was downregulated in the whole biopsy samples, but not detectable in LE, GE, and stroma suggesting main expression in blood vessels. PGF2α) secreted from the endometrium is

a major luteolysin. Furthermore, there is evidence that the mare has like some other mammals an PGF2a auto-amplification system in which PGF2a can stimulate its own production and *PTGFR* downregulation is uncoupling this system and thereby contributing to prevention of luteolysis (de Ruijter-Villani *et al.* 2015; Kozai *et al.* 2016).

In agreement with our recent study, the mRNA for oxytocin receptor (*OXTR*) was upregulated in whole biopsy samples. The data from LCM samples revealed expression in all compartments and upregulation in GE (medium confidence). Estrogen receptor 1 mRNA (*ESR1*) was slightly downregulated for the whole biopsy dataset (FDR 0.034) confirming our and other recent findings (Klein *et al.* 2010; Merkl *et al.* 2010). However, the LCM data revealed expression in LE, GE, and stroma with increased expression in stroma collected from Day 12 pregnant mares. Due to the relatively high variation of expression between biological replicates, this has to be further confirmed. Not only for the mare, *OXTR* and *ESR1* have been suggested to play a major role in the oxytocin-PGF2a feedback loop involved in regulation of luteolysis (de Ruijter-Villani *et al.* 2015).

In conclusion, this study revealed new and important spatial information about endometrial gene expression during the phase of initial recognition of pregnancy in the mare using laser capture microdissection to specifically study LE, GE, and stromal compartments. The results indicate a direct and/or indirect (via LE) induction of angiogenesis and blood vessel development by the presence of the conceptus. Expression changes of immune-related genes suggest a specific spatial regulation of the immune system. Differential expression of many growth factors involved in embryo-maternal interaction and intraendometrial communication controlling cell differentiation and endometrial remodeling were identified. Finally, expression of genes involved in PG metabolism and signaling indicates transcriptional regulation of genes functioning in production of PG precursor, PG transport, and PG receptors, specifically PTGFR in context of prevention of luteolysis.

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590

591 **Conflicts of Interest**

592 The authors declare no conflicts of interest.

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Figure legends

Figure 1: Pairwise distance heatmap (Geneplotter) (A) and principal component analysis (Multidimensional Scaling Plot, EdgeR) (B) for LCM data sets. P: pregnant, C: cyclic control, LE: luminal epithelium, GE: glandular epithelium, S: stromal areas, last letter: initial of the mare's name.

Figure 2: Venn diagram for comparison of detectable genes in the RNA-Seq analyses of endometrial biopsies and cell type-specific samples isolated by Laser Capture Microdissection. LE: luminal epithelium, GE: glandular epithelium.

Figure 3: Venn diagram for comparison of differentially expressed genes in different endometrial compartments (LCM samples) and complete endometrial biopsy. LE: luminal epithelium, GE: glandular epithelium.

Figure 4: Venn diagram for comparison of differentially expressed genes in different endometrial compartments (LCM samples) in consideration of up- and downregulation in Day 12 pregnant vs. cyclic endometrium. LE: luminal epithelium, GE: glandular epithelium.

Supplemental Figure 1: Hierarchical cluster analysis of differentialy expressed genes in luminal epithelium (LE). Mean-centered expression values (log_2 scale) were calculated and used for hierarchical cluster analysis (MeV_4_8 v10.2). GE: glandular epithelium, S: stromal areas, P: pregnant, C: cyclic control, last letter of sample ID: initial of mare's name.

Supplemental Figure 2: Hierarchical cluster analysis of differentialy expressed genes in glandular epithelium (GE). Mean-centered expression values (log_2 scale) were calculated and used for hierarchical cluster analysis (MeV_4_8 v10.2). LE: luminal epithelium, S: stromal areas, P: pregnant, C: cyclic control, last letter of sample ID: initial of mare's name.

Supplemental Figure 3: Hierarchical cluster analysis of differentialy expressed genes in stroma. Mean-centered expression values (log_2 scale) were calculated and used for hierarchical cluster analysis (MeV_4_8 v10.2). LE: luminal epithelium, GE: glandular epithelium, S: stromal areas, P: pregnant, C: cyclic control, last letter of sample ID: initial of mare's name.

Tables

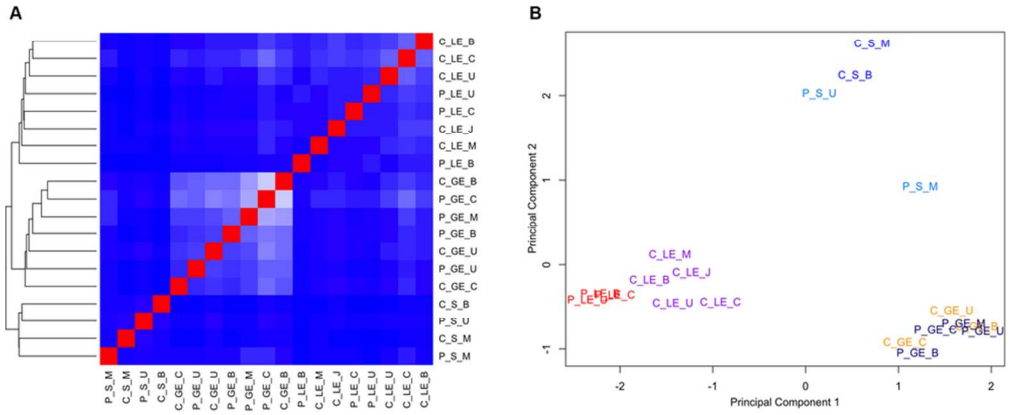
Table 1: Overview of obtained differentially expressed genes for day 12 of pregnancy in comparison to day 12 of the estrous cycle

Sample	Higher in pregnancy	Lower in pregnancy	Total
Biopsy	234	139	373
Luminal epithelium	94	71	165
Glandular epithelium	17	25	42
Stroma	138	16	154

962 **Figures**

963

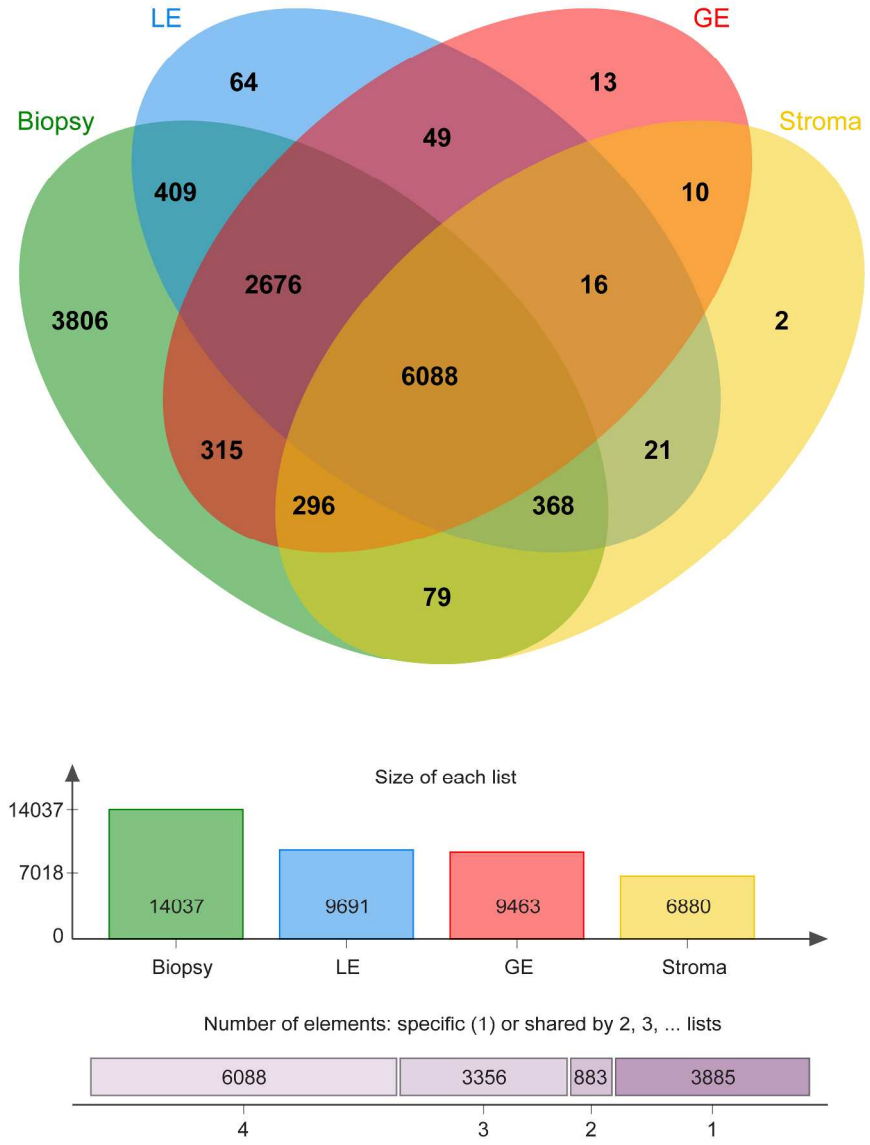
Figure 1



Pairwise distance heatmap (Geneplotter) (A) and principal component analysis (Multidimensional Scaling Plot, EdgeR) (B) for LCM data sets. P: pregnant, C: cyclic control, LE: luminal epithelium, GE: glandular epithelium, S: stromal areas, last letter: initial of the mare's name.

84x38mm (300 x 300 DPI)

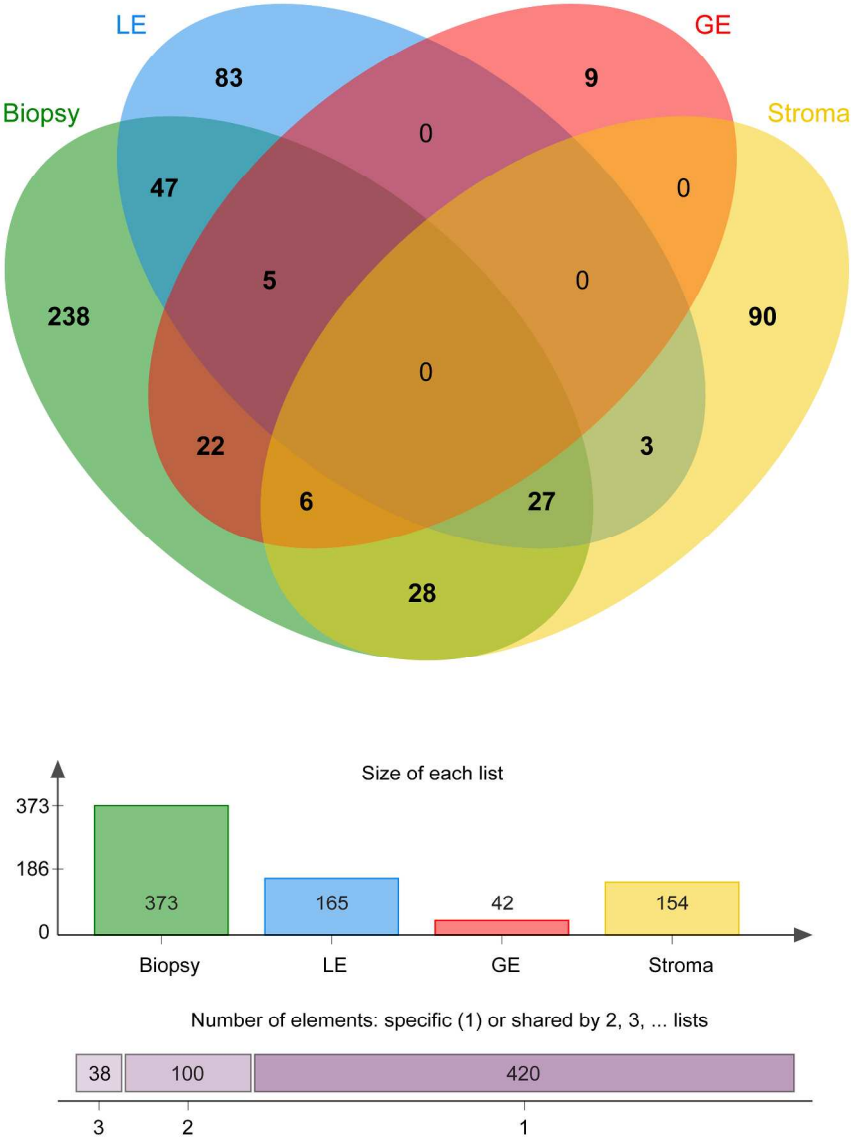
Figure 2



Venn diagram for comparison of detectable genes in the RNA-Seq analyses of endometrial biopsies and cell type-specific samples isolated by Laser Capture Microdissection. LE: luminal epithelium, GE: glandular epithelium.

230x322mm (300 x 300 DPI)

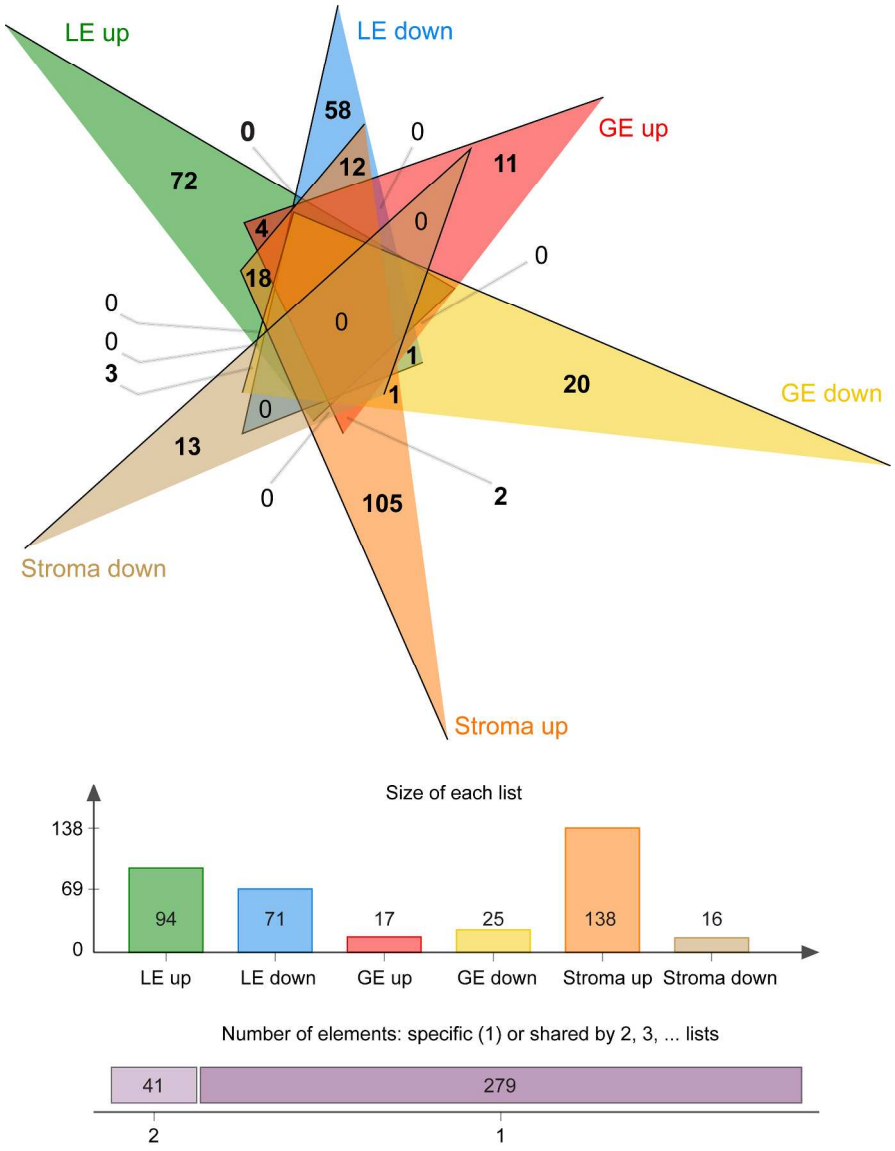
Figure 3



Venn diagram for comparison of differentially expressed genes in different endometrial compartments (LCM samples) and complete endometrial biopsy. LE: luminal epithelium, GE: glandular epithelium.

229x324mm (300 x 300 DPI)

Figure 4



Venn diagram for comparison of differentially expressed genes in different endometrial compartments (LCM samples) in consideration of up- and downregulation in Day 12 pregnant vs. cyclic endometrium. LE: luminal epithelium, GE: glandular epithelium.

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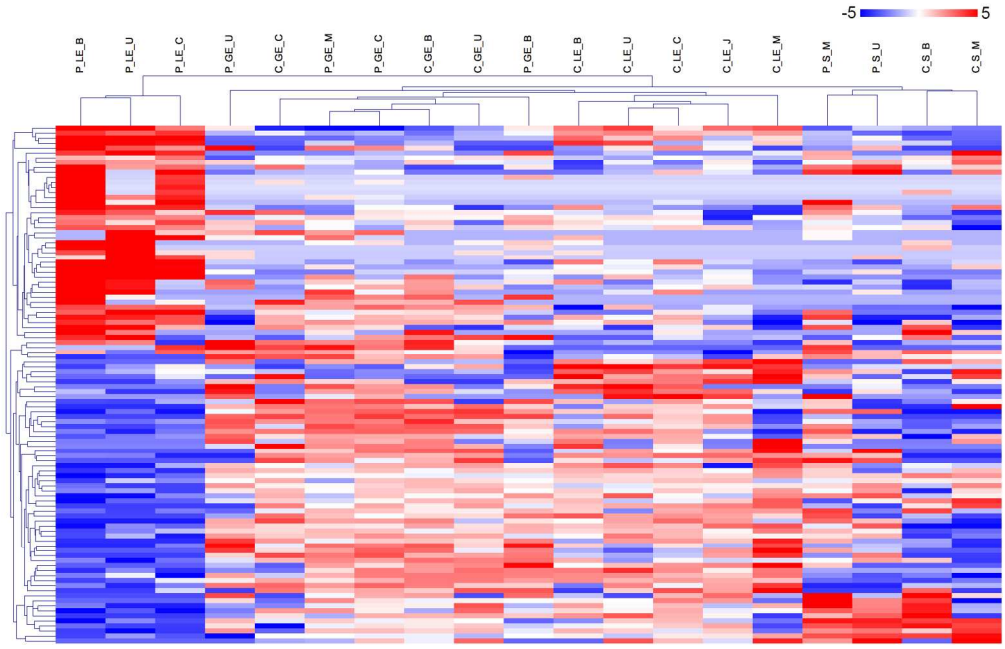
Supplemental Figure 1



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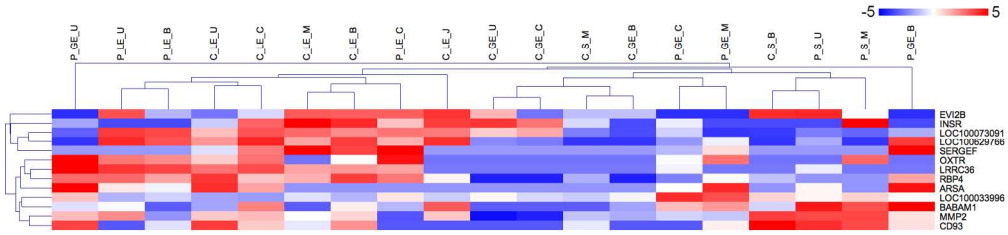
Supplemental Figure 2



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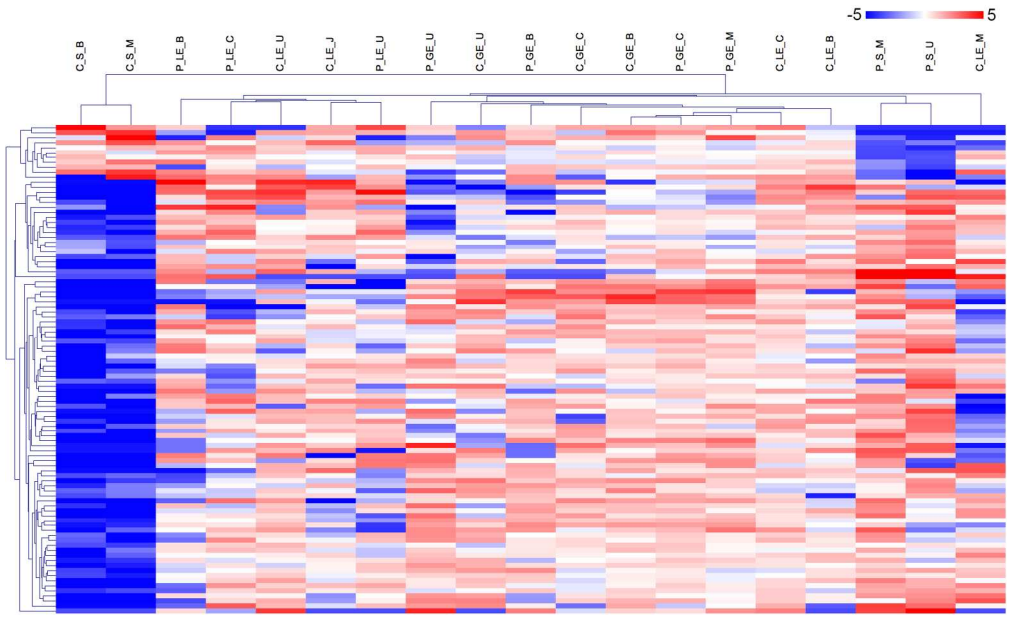
Supplemental Figure 3



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Supplemental Table 1: Serum progesterone concentrations

Mare	C	M	U	B	J
Day	control cycle P4 (ng/ml)				
-2	1.0	0.6	0.3	0.2	0.3
-1	2.9	0.6	1.4	0.3	0.8
0	18.7	46.6	18.6	19.5	19.5
4	34.8	35.7	20.4	27.0	23.7
8	26.2	36.4	23.6	26.8	25.8
9	30.3	44.6	19.4	22.4	23.3
10	22.3	26.1	22.6	25.5	23.5
11	23.0	21.9	17.4	26.2	23.8
12	26.1	36.2	21.9	31.4	23.5
Day	pregnancy cycle P4 (ng/ml)				
-2	0.2	0.4	0.3	0.3	0.7
-1	0.3	0.8	0.3	0.3	0.9
0	57.4	18.3	28.0	22.5	74.3
4	92.9	38.1	40.7	23.9	70.5
8	100.4	27.0	22.6	32.4	68.6
9	99.2	32.3	57.2	26.6	80.6
10	81.4	30.4	43.2	29.5	61.4
11	45.8	28.8	51.0	35.2	73.4
12	79.5	32.4	40.1	28.5	75.9

Supplemental Table 4: Comparison of functional terms between LCM samples and complete e

Group	Category	Term	LE	Count
Blood vessel/vasculature development	GOTERM_BP_FAT	GO:0001568 blood vessel development		10
	GOTERM_BP_FAT	GO:0001525 angiogenesis		8
	GOTERM_BP_FAT	GO:0045765 regulation of angiogenesis		4
	GOTERM_BP_FAT	GO:0045766 positive regulation of angiogenesis		4
	GOTERM_BP_FAT	GO:0001944 vasculature development		11
	GOTERM_BP_FAT	GO:1901342 regulation of vasculature development		4
	GOTERM_BP_FAT	GO:0001570 vasculogenesis		2
	GOTERM_BP_FAT	GO:0003158 endothelium development		3
	GOTERM_BP_FAT	GO:0045446 endothelial cell differentiation		2
	GOTERM_BP_FAT	GO:0001935 endothelial cell proliferation		1
	GOTERM_BP_FAT	GO:0043542 endothelial cell migration		3
Response to...	GOTERM_BP_FAT	GO:0070482 response to oxygen levels		7
	GOTERM_BP_FAT	GO:0080134 regulation of response to stress		19
	GOTERM_BP_FAT	GO:0032101 regulation of response to external stimulus		11
	GOTERM_BP_FAT	GO:0032102 negative regulation of response to external stimulus		7
	GOTERM_BP_FAT	GO:0032526 response to retinoic acid		3
Immune response	GOTERM_BP_FAT	GO:0006955 immune response		7
	GOTERM_BP_FAT	GO:0002682 regulation of immune system process		12
	GOTERM_BP_FAT	GO:0006954 inflammatory response		10
	GOTERM_BP_FAT	GO:0050727 regulation of inflammatory response		7
	GOTERM_BP_FAT	GO:0045087 innate immune response		4
	GOTERM_BP_FAT	GO:0006956 complement activation		2
	GOTERM_BP_FAT	GO:0030449 regulation of complement activation		2
	GOTERM_BP_FAT	GO:0030193 regulation of blood coagulation		4
	GOTERM_BP_FAT	GO:0030195 negative regulation of blood coagulation		4
	GOTERM_BP_FAT	GO:0006959 humoral immune response		3
	GOTERM_BP_FAT	GO:0016477 cell migration		11
	GOTERM_BP_FAT	GO:0006935 chemotaxis		5
	GOTERM_BP_FAT	GO:0030595 leukocyte chemotaxis		3
	GOTERM_BP_FAT	GO:0050900 leukocyte migration		5
	GOTERM_BP_FAT	GO:0002685 regulation of leukocyte migration		2
	GOTERM_BP_FAT	GO:0002694 regulation of leukocyte activation		6
Cell proliferattion, cell death	GOTERM_BP_FAT	GO:0008283 cell proliferation		20
	GOTERM_BP_FAT	GO:0042127 regulation of cell proliferation		16
	GOTERM_BP_FAT	GO:0008284 positive regulation of cell proliferation		12
	GOTERM_BP_FAT	GO:0050673 epithelial cell proliferation		6
	GOTERM_BP_FAT	GO:0030855 epithelial cell differentiation		4
	GOTERM_BP_FAT	GO:0008219 cell death		21
	GOTERM_BP_FAT	GO:0012501 programmed cell death		18
	GOTERM_BP_FAT	GO:0043067 regulation of programmed cell death		14
	GOTERM_BP_FAT	GO:0060548 negative regulation of cell death		15
ion,	GOTERM_BP_FAT	GO:0009893 positive regulation of metabolic process		13

Metabolic process, glycosylati phosphorylation	GOTERM_BP_FAT	GO:0051246 regulation of protein metabolic process	20
	GOTERM_BP_FAT	GO:0051241 negative regulation of multicellular organismal process	14
	GOTERM_BP_FAT	GO:0019216 regulation of lipid metabolic process	1
	GOTERM_BP_FAT	GO:0009101 glycoprotein biosynthetic process	9
	GOTERM_BP_FAT	GO:0016266 O-glycan processing	4
	GOTERM_BP_FAT	GO:0001934 positive regulation of protein phosphorylation	6
	GOTERM_BP_FAT	GO:0045859 regulation of protein kinase activity	5
	GOTERM_BP_FAT	GO:0045860 positive regulation of protein kinase activity	3
Cell adhesion, junction	GOTERM_BP_FAT	GO:0007155 cell adhesion	17
	GOTERM_BP_FAT	GO:0016337 single organismal cell-cell adhesion	11
	GOTERM_BP_FAT	GO:0098609 cell-cell adhesion	13
	GOTERM_BP_FAT	GO:0034329 cell junction assembly	1
	GOTERM_BP_FAT	GO:0034330 cell junction organization	1
	GOTERM_BP_FAT	GO:0045216 cell-cell junction organization	
Extracellular, cell surface	GOTERM_CC_FAT	GO:0005576 extracellular region	44
	GOTERM_CC_FAT	GO:0031012 extracellular matrix	13
	GOTERM_CC_FAT	GO:0005578 proteinaceous extracellular matrix	10
	GOTERM_CC_FAT	GO:0005615 extracellular space	18
	GOTERM_BP_FAT	GO:0061448 connective tissue development	8
	GOTERM_CC_FAT	GO:0031226 intrinsic component of plasma membrane	17
	GOTERM_CC_FAT	GO:0009986 cell surface	11
	GOTERM_CC_FAT	GO:0016324 apical plasma membrane	8
Secretion, extracellular vesicles	GOTERM_CC_FAT	GO:0060205 cytoplasmic membrane-bounded vesicle lumen	3
	GOTERM_CC_FAT	GO:1903561 extracellular vesicle	32
	GOTERM_CC_FAT	GO:0016023 cytoplasmic, membrane-bounded vesicle	13
	GOTERM_BP_FAT	GO:0016192 vesicle-mediated transport	12
	GOTERM_BP_FAT	GO:0046903 secretion	15
	GOTERM_CC_FAT	GO:0099503 secretory vesicle	6
	GOTERM_CC_FAT	GO:0030141 secretory granule	6
Diverse terms	GOTERM_BP_FAT	GO:0006939 smooth muscle contraction	1
	GOTERM_CC_FAT	GO:0030485 smooth muscle contractile fiber	
	GOTERM_BP_FAT	GO:0051051 negative regulation of transport	9
	GOTERM_MF_FAT	GO:0005520 insulin-like growth factor binding	2
	SP_COMMENT	similarity:Belongs to the small heat shock protein (HSP20) family	3
	UCSC_TFBS	IRF1	17

endometrial biopsy

FE	P-Value	FDR	Entrez Gene IDs	GE	Count	FE	P-Value	FDR	Entrez Gene IDs	S
2.97	0.0058	9.76	4856 302 51129 358 22		1	1.80	1.0000	100.00	4313	
3.48	0.0075	12.39	4856 302 51129 358 22		1	2.65	1.0000	100.00	4313	
3.51	0.1026	85.21	51129 358 947 3315							
6.41	0.0236	34.35	51129 358 947 3315							
3.07	0.0027	**4.64**	5784 4856 302 51129 3		1	1.69	1.0000	100.00	4313	
3.21	0.1257	90.65	51129 358 947 3315							
4.39	0.3647	99.97	7477 947							
3.93	0.1738	96.55	6781 7477 947							
3.11	0.4736	100.00	6781 7477							
1.60	1.0000	100.00	947							
3.29	0.2273	98.94	6781 4856 3315							
3.68	0.0111	17.91	1410 6781 51129 4582		3	9.63	0.0327	40.50	4313 4582 5021	
2.45	0.0005	**0.81**	1410 4856 8870 302 74		5	3.95	0.0245	32.15	4313 9180 29086 4582	
3.30	0.0016	**2.76**	90355 4856 9180 302 5		1	1.87	1.0000	100.00	9180	
5.65	0.0014	**2.37**	90355 4856 302 5327 2							
5.31	0.1070	86.43	4582 358 7477		2	21.79	0.0820	73.74	5950 4582	
0.97	0.7253	100.00	1113 710 26999 27242		1	0.87	1.0000	100.00	4313	
1.73	0.0796	76.86	3549 4856 57864 2843		2	1.78	0.6624	100.00	5950 4313	
3.96	0.0008	**1.44**	90355 4856 9180 4968		1	2.51	1.0000	100.00	9180	
5.35	0.0018	**3.13**	90355 4856 9180 2843		1	4.88	1.0000	100.00	9180	
0.97	0.7808	100.00	1113 710 1604 4486		1	1.50	1.0000	100.00	4313	
8.78	0.2027	98.16	710 1604							
14.43	0.1287	91.21	710 1604							
10.36	0.0064	10.73	302 5327 710 947							
22.44	0.0007	**1.16**	302 5327 710 947							
4.97	0.1198	89.47	710 27242 1604							
1.67	0.1135	88.07	90355 6781 4856 3486							
2.02	0.2287	98.98	4856 284340 1113 331							
4.27	0.1530	94.66	4856 284340 1113							
2.95	0.0863	79.67	4856 2650 284340 111							
3.26	0.4577	100.00	4856 284340							
2.78	0.0621	67.74	3549 57864 4345 2724							
2.04	0.0028	**4.90**	5784 6781 4856 302 82		4	2.49	0.1801	95.51	5950 4313 9180 22809	
2.04	0.0093	15.26	5784 4856 302 826 627		4	3.12	0.1091	83.56	5950 4313 9180 22809	
3.06	0.0016	**2.73**	1397 3549 9180 302 82		2	3.12	0.4564	99.99	4313 9180	
3.12	0.0412	52.40	3549 4856 3486 2254 9							
1.45	0.5152	100.00	6781 4582 7477 947		1	2.20	1.0000	100.00	4582	
1.82	0.0074	12.34	1410 4856 8870 64114		2	1.06	0.8490	100.00	4582 22809	
1.65	0.0363	47.94	1410 8870 64114 3315		2	1.12	0.8319	100.00	4582 22809	
1.70	0.0609	67.00	1410 8870 64114 3315		2	1.47	0.7360	100.00	4582 22809	
2.90	0.0005	**0.87**	1410 4856 8870 64114		2	2.34	0.5598	100.00	4582 22809	
0.73	0.9537	100.00	302 2254 284340 7477		4	1.37	0.5291	100.00	29086 4582 51339 228	

1.33	0.1498	94.30	1410 653145 302 2254	2	0.81	0.9201	100.00	4582 51339
2.78	0.0012	**2.17**	6781 4856 302 8111 11	2	2.43	0.5453	100.00	5950 5021
0.59	1.0000	100.00	8013					
4.33	0.0010	**1.74**	4585 64772 25825 4582	2	5.87	0.2744	99.33	4582 94025
13.93	0.0028	**4.74**	4585 4582 2650 93010	2	45.19	0.0404	47.46	4582 94025
1.31	0.4790	100.00	302 3486 2254 7477 28					
1.21	0.5903	100.00	5997 7477 284340 3315					
1.13	0.7415	100.00	7477 284340 4486					
1.85	0.0166	25.57	4856 302 7477 3315 94	3	2.01	0.4116	99.97	4582 94025 22918
3.11	0.0024	**4.20**	3549 4856 57864 7477	1	1.78	1.0000	100.00	22918
2.05	0.0210	31.26	4856 302 7477 947 331	1	0.97	1.0000	100.00	22918
0.70	1.0000	100.00	3914					
0.59	1.0000	100.00	3914					
1.81	0.0000	**0.01**	1410 4856 64114 826 2	7	1.88	0.0971	66.86	5950 4313 55503 4582
4.55	0.0000	**0.03**	1397 4585 4856 302 51	2	4.57	0.3374	98.83	4313 22918
5.98	0.0000	**0.05**	4585 4856 302 51129 3	1	3.86	1.0000	100.00	4313
3.19	0.0000	**0.04**	6781 302 2254 7477 28	6	7.02	0.0006	**0.70**	5950 4313 4582 94025
6.36	0.0002	**0.41**	6781 3549 4856 2254 7					
2.72	0.0003	**0.45**	2681 8111 947 27242 4	4	4.27	0.0503	42.74	9180 55503 4582 5021
2.96	0.0035	**4.44**	1410 302 25825 57864	2	3.61	0.4073	99.65	410 22918
5.08	0.0009	**1.15**	6781 9180 3775 4582 3	3	12.22	0.0207	20.20	9180 4582 5021
6.18	0.0828	67.51	7076 710 3959					
1.64	0.0025	**3.26**	1410 826 64114 153201	6	2.01	0.1174	74.07	5950 55503 4582 94025
1.78	0.0551	52.17	302 7477 1113 26509 5	2	1.78	0.6622	100.00	410 22918
1.36	0.2557	99.45	302 55503 7076 1113 7	2	1.37	0.7615	100.00	55503 22918
2.82	0.0006	**1.14**	6781 4856 302 8111 11	4	4.62	0.0422	49.02	5950 26297 55503 5021
2.59	0.0788	65.66	5327 7076 1113 710 58	1	2.80	1.0000	100.00	410
3.31	0.0332	35.53	5327 7076 1113 710 58	1	3.61	1.0000	100.00	410
3.16	1.0000	100.00	5997	1	20.34	1.0000	100.00	5021
3.97	0.0017	**3.01**	1410 6781 4856 302 11	2	5.42	0.2934	99.56	26297 5021
13.11	0.1405	88.15	4856 3486					
78.50	0.0005	**0.75**	1410 26353 3315					
0.73	0.9698	100.00	84557 5784 653145 230					

Count	FE	P-Value	FDR	Entrez Gene IDs	Biop	Count	FE	P-Value	FDR	Entrez Gene IDs
13	2.34	0.0089	14.89	641700 79812 4856 5		40	4.01	0.0000	**0.00**	1003 4856 59 688 5333
12	3.14	0.0014	**2.51**	641700 79812 3685 4		35	4.90	0.0000	**0.00**	4856 688 5333 2702 22
6	3.12	0.0418	53.84	641700 79812 51129		20	5.21	0.0000	**0.00**	79812 641700 1893 196
3	2.89	0.2760	99.71	51129 947 7057		11	5.01	0.0001	**0.13**	1893 718 146850 51129
13	2.21	0.0137	22.08	641700 79812 4856 5		44	4.17	0.0000	**0.00**	1003 4856 59 688 5333
6	2.84	0.0588	66.59	641700 79812 51129		21	4.96	0.0000	**0.00**	79812 641700 1893 749
1	1.46	1.0000	100.00	947		9	7.33	0.0000	**0.06**	51162 7498 7075 1969
3	2.33	0.3661	99.97	5175 1901 947		12	6.25	0.0000	**0.01**	1003 6781 7498 2702 5
2	1.83	0.6665	100.00	5175 1901		9	5.48	0.0002	**0.44**	1003 6781 7498 5175 7
3	2.70	0.3020	99.85	947 7057 1739		14	7.10	0.0000	**0.00**	1893 7498 1969 3791 8
6	3.87	0.0187	28.91	79812 4856 5175 270		12	4.50	0.0001	**0.14**	79812 6781 4856 6273
7	2.32	0.0788	77.36	9124 4194 51129 532		20	3.68	0.0000	**0.00**	1410 6781 6649 3708 5
23	1.83	0.0057	9.81	4856 6647 4214 4216		53	2.38	0.0000	**0.00**	1410 4856 7498 5734 3
15	2.76	0.0009	**1.67**	5919 4856 3827 1901		39	3.13	0.0000	**0.00**	2621 4681 286133 4856
7	3.49	0.0146	23.37	4856 5327 3827 710		14	3.03	0.0008	**1.45**	4681 4856 5734 3827 2
						9	4.91	0.0005	**0.93**	5950 3490 7075 3485 4
15	1.29	0.2589	99.56	8976 4684 4214 2099		48	1.79	0.0001	**0.18**	2621 8519 81035 9572
16	1.43	0.1432	93.90	5919 4856 6647 8976		53	2.22	0.0000	**0.00**	5950 5919 4856 5734 6
10	2.48	0.0187	28.92	4856 5919 6366 3827		34	3.09	0.0000	**0.00**	4856 5919 5734 3827 9
7	3.37	0.0171	26.76	4856 720 710 27086		19	3.50	0.0000	**0.02**	4856 5734 284340 5648
10	1.51	0.2134	98.70	6366 4684 4214 3107		32	2.21	0.0001	**0.10**	3594 1755 8519 81035
2	5.16	0.3220	99.91	720 710		12	5.93	0.0000	**0.01**	716 2 28834 718 715 72
2	8.73	0.2051	98.43	720 710		7	11.90	0.0000	**0.04**	2 718 722 720 710 730
6	8.51	0.0006	**1.10**	5327 3827 710 947 7		7	4.82	0.0033	6.04	286133 5327 3827 710
5	14.94	0.0003	**0.51**	5327 3827 710 947 7		5	6.28	0.0080	14.08	5327 3827 710 947 705
2	1.96	0.6419	100.00	720 710		15	3.50	0.0001	**0.20**	1755 100423062 5648 2
19	1.76	0.0199	30.49	79812 2018 5919 485		52	2.54	0.0000	**0.00**	4856 5919 5734 6422 2
13	3.28	0.0005	**0.98**	1808 641700 3685 48		27	2.83	0.0000	**0.01**	2621 4681 5919 4856 3
5	4.24	0.0293	41.67	4856 5919 6366 1901		14	4.17	0.0000	**0.06**	2621 4681 6387 79148
10	3.59	0.0017	**3.09**	3685 4856 5919 8976		24	3.69	0.0000	**0.00**	4681 2621 5919 4856 1
5	4.98	0.0173	27.04	4856 5919 8976 6366		14	5.19	0.0000	**0.01**	8784 2621 4681 6387 7
4	1.14	0.6815	100.00	6647 6366 7057 1739		20	2.47	0.0005	**0.96**	3594 2621 8660 857 10
19	1.22	0.2806	99.74	2018 4856 4194 5768		68	2.11	0.0000	**0.00**	1003 5950 4856 2006 1
18	1.45	0.1051	86.58	3714 4856 2065 4194		62	2.31	0.0000	**0.00**	1003 5950 4856 1001 6
10	1.60	0.1682	96.43	3685 1901 55023 259		32	2.18	0.0001	**0.13**	2621 1001 688 2254 64
8	2.54	0.0371	49.56	4856 1749 27086 209		24	4.00	0.0000	**0.00**	4856 1001 1893 7498 9
13	2.84	0.0019	**3.40**	3714 6647 59 6876 5		26	2.67	0.0000	**0.03**	1003 1755 3714 6781 2
29	1.58	0.0124	20.17	4856 5618 3827 2708		60	1.77	0.0000	**0.02**	1410 4856 5166 7498 3
27	1.54	0.0210	31.89	5618 3827 27086 209		52	1.62	0.0005	**0.90**	1410 5166 7498 3827 4
22	1.67	0.0190	29.28	641700 6647 2065 41		45	1.84	0.0001	**0.17**	2621 1410 5166 7498 5
19	2.31	0.0012	**2.16**	4856 6647 2065 4194		35	2.26	0.0000	**0.03**	2621 1410 4856 5166 5
44	1.56	0.0014	**2.43**	5919 59 5618 83939		66	1.25	0.0361	50.15	5919 1001 688 6775 74

40	1.67	0.0007	**1.35**	5919 5618 83939 382	69	1.59	0.0001	**0.14**	1410 5919 7498 3827 2
15	1.83	0.0326	45.08	79812 641700 4856 6	38	2.16	0.0000	**0.03**	2621 5950 4856 1001 2
3	1.17	0.7291	100.00	5919 6647 6366	15	3.02	0.0005	**0.90**	5447 5919 5166 9572 8
6	1.91	0.2007	98.26	6366 2681 56886 513	12	1.96	0.0440	57.29	8693 117248 4585 493
					7	6.74	0.0006	**1.07**	8693 117248 4585 458
20	2.67	0.0001	**0.24**	5919 6647 2065 5618	34	2.21	0.0000	**0.05**	2621 5919 5618 7498 2
17	2.59	0.0007	**1.34**	6647 2065 5618 4214	20	1.58	0.0493	61.56	2621 10253 5618 7477
13	3.04	0.0011	**1.92**	81669 6647 2065 636	15	1.85	0.0334	47.42	2621 10253 5618 2843
27	1.81	0.0029	5.12	4856 4684 5618 8393	80	2.72	0.0000	**0.00**	1003 4856 1001 800 57
12	2.08	0.0277	39.82	3685 3714 4856 6647	33	2.63	0.0000	**0.00**	1003 2621 4856 1001 5
17	1.66	0.0458	57.20	4856 3714 6647 5618	43	2.14	0.0000	**0.01**	2621 1003 4856 1001 5
2	0.85	0.9063	100.00	7057 1739	13	3.91	0.0001	**0.24**	1003 3918 2702 3914 5
2	0.72	0.9402	100.00	7057 1739	16	3.71	0.0000	**0.05**	1003 1001 2702 3791 3
2	0.83	0.9128	100.00	7057 1739	13	3.45	0.0004	**0.78**	1003 1001 2702 55679
56	1.47	0.0008	**1.11**	192668 4856 5919 10	158	1.77	0.0000	**0.00**	1410 192668 5950 2006
13	2.74	0.0025	**3.37**	79812 5919 4856 664	48	4.68	0.0000	**0.00**	4856 2006 5919 119587
7	2.50	0.0592	56.67	79812 4856 6366 511	34	4.87	0.0000	**0.00**	4856 2006 2254 1690 7
19	2.10	0.0035	**4.63**	79812 6647 59 2065	83	2.97	0.0000	**0.00**	5950 7498 119587 9402
4	1.94	0.3359	99.94	4856 59 960 947	14	3.43	0.0002	**0.45**	4256 5447 4856 6781 5
12	1.27	0.3387	99.66	3685 3714 9375 2065	55	1.67	0.0001	**0.20**	2702 5734 5021 943 87
17	2.88	0.0002	**0.30**	5618 4684 5175 1901	36	2.43	0.0000	**0.00**	1003 286133 1410 5618
2	0.77	0.9274	100.00	2065 947	15	2.65	0.0017	**2.32**	55107 6781 114902 502
5	6.38	0.0073	9.52	5919 5768 3827 710	13	6.40	0.0000	**0.00**	2621 2 3481 5919 1893
43	1.40	0.0122	15.54	192668 5919 59 4684	106	1.94	0.0000	**0.00**	5950 192668 1410 9402
22	1.91	0.0045	5.98	5919 6647 8976 5175	41	1.79	0.0003	**0.47**	117248 2621 5919 1149
18	1.26	0.2504	99.46	5919 6647 8976 5175	45	1.74	0.0003	**0.57**	2621 286133 9980 5919
16	1.90	0.0196	30.17	5919 4856 6647 2065	49	2.58	0.0000	**0.00**	2621 5950 4856 5919 1
10	2.74	0.0103	13.18	5919 6647 54843 517	23	2.55	0.0001	**0.15**	2621 1755 5919 1893 5
9	3.09	0.0081	10.60	5919 6647 5175 5768	22	3.18	0.0000	**0.01**	2621 1755 5919 1893 5
6	12.16	0.0001	**0.19**	5997 6647 59 4629 2	5	3.44	0.0572	67.17	5997 59 5021 4629 255
3	53.62	0.0010	**1.37**	59 5175 255743	3	38.77	0.0022	**2.97**	59 5175 255743
8	2.19	0.0704	73.29	3685 4856 2065 2593	15	1.93	0.0246	37.50	2621 1410 6781 4856 5
2	7.45	0.2358	97.94	3685 4856	8	16.88	0.0000	**0.00**	3490 4856 3491 3484 3
					2	12.44	0.1492	93.27	1410 26353
51	1.42	0.0039	**4.69**	2099 8639 947 11314	77	1.23	0.0327	33.47	1001 688 6775 800 982

Supplemental Table 5: Genes assigned to selected signaling pathways

Human Entrez Gene ID	Symbol	Description	LE	GE
4363	ABCC1	ATP binding cassette subfamily C member 1	up	up
1645	AKR1C1	aldo-keto reductase family 1 member C1		
301	ANXA1	annexin A1		
302	ANXA2	annexin A2	up	
960	CD44	CD44 molecule (Indian blood group)	down	
6387	CXCL12	C-X-C motif chemokine ligand 12		
1649	DDIT3	DNA damage inducible transcript 3	down	
201562	HACD2	3-hydroxyacyl-CoA dehydratase 2		
4313	MMP2	matrix metallopeptidase 2		up
1728	NQO1	NAD(P)H quinone dehydrogenase 1		down
10630	PDPN	podoplanin		
5320	PLA2G2A	phospholipase A2 group IIA	down	
255189	PLA2G4F	phospholipase A2 group IVF		up?
5734	PTGER4	prostaglandin E receptor 4		
5737	PTGFR	prostaglandin F receptor		
5740	PTGIS	prostaglandin I2 synthase		
22949	PTGR1	prostaglandin reductase 1	up	
6422	SFRP1	secreted frizzled related protein 1		
6578	SLCO2A1	solute carrier organic anion transporter family member 2A1		
7076	TIMP1	TIMP metallopeptidase inhibitor 1	up	
3371	TNC	tenascin C		
196883	ADCY4	adenylate cyclase 4		
10000	AKT3	AKT serine/threonine kinase 3	down	
783	CACNB2	calcium voltage-gated channel auxiliary subunit beta 2		
27091	CACNG5	calcium voltage-gated channel auxiliary subunit gamma 5		
148327	CREB3L4	cAMP responsive element binding protein 3 like 4		
1910	EDNRB	endothelin receptor type B		
2099	ESR1	estrogen receptor 1		
3708	ITPR1	inositol 1,4,5-trisphosphate receptor type 1		
4240	MFGE8	milk fat globule-EGF factor 8 protein		
10398	MYL9	myosin light chain 9		
4638	MYLK	myosin light chain kinase	down	
5021	OXTR	oxytocin receptor		up
5295	PIK3R1	phosphoinositide-3-kinase regulatory subunit 1	down	
146850	PIK3R6	phosphoinositide-3-kinase regulatory subunit 6		
5563	PRKAA2	protein kinase AMP-activated catalytic subunit alpha 2		
5618	PRLR	prolactin receptor		
5997	RGS2	regulator of G-protein signaling 2	up	
8660	IRS2	insulin receptor substrate 2		

4214	MAP3K1	mitogen-activated protein kinase kinase kinase 1	
4792	NFKBIA	NFKB inhibitor alpha	
5879	RAC1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)	
57521	RPTOR	regulatory associated protein of MTOR complex 1	down
6775	STAT4	signal transducer and activator of transcription 4	

LE: luminal epithelium; GE: glandular epithelium; up: higher expression in endometrium of day 12 pregnant mares; down: lower express

Stroma	Biopsy	hsa01040 Biosynthesis of unsaturated fatty acids	hsa00590 Arachidonic acid metabolism	WP98_45273 PG synthesis and regulation	Entrez Gene "prostaglandin"	Entrez Gene "prostaglandin transporter"	hsa00140 Steroid hormone biosynthesis	hsa04915 Estrogen signaling pathway	WP712_48214 Estrogen signaling	hsa04921 Oxytocin signaling pathway	hsa04917 Prolactin signaling pathway	WP585 Interferon type I signaling pathways
	up					x						
	up				x		x					
down				x	x							
				x								
up	up			*	x							
	up			*				*				
				*	x							
up		x										
					x			x				
	down				x							
	up			*	x							
			x									
	up		x		x					x		
	up			x	x							
	down			x	x							
	up		x	x	x							
	up			*	x							
	up			*	x			*				
	up				x	x						
					x							
	up			*	x			*				
	up							x		x		
								x			x	
	down									x		
	up									x		
	down							x				
	down			x				*				
up					x			x	x		x	
	up							x		x		
	up										*	
up	up									x		
										x		
	up			*						x		
								x			x	x
	up									x		
	down				x					x		
up	up										x	
up	up									x		
	up											x

up		x
up		
	up	x
		x
	up	x

sion in endometrium of day 12 pregnant mares; *assigned based on literature search

WP195 IL-1 signaling pathway

x

x
x